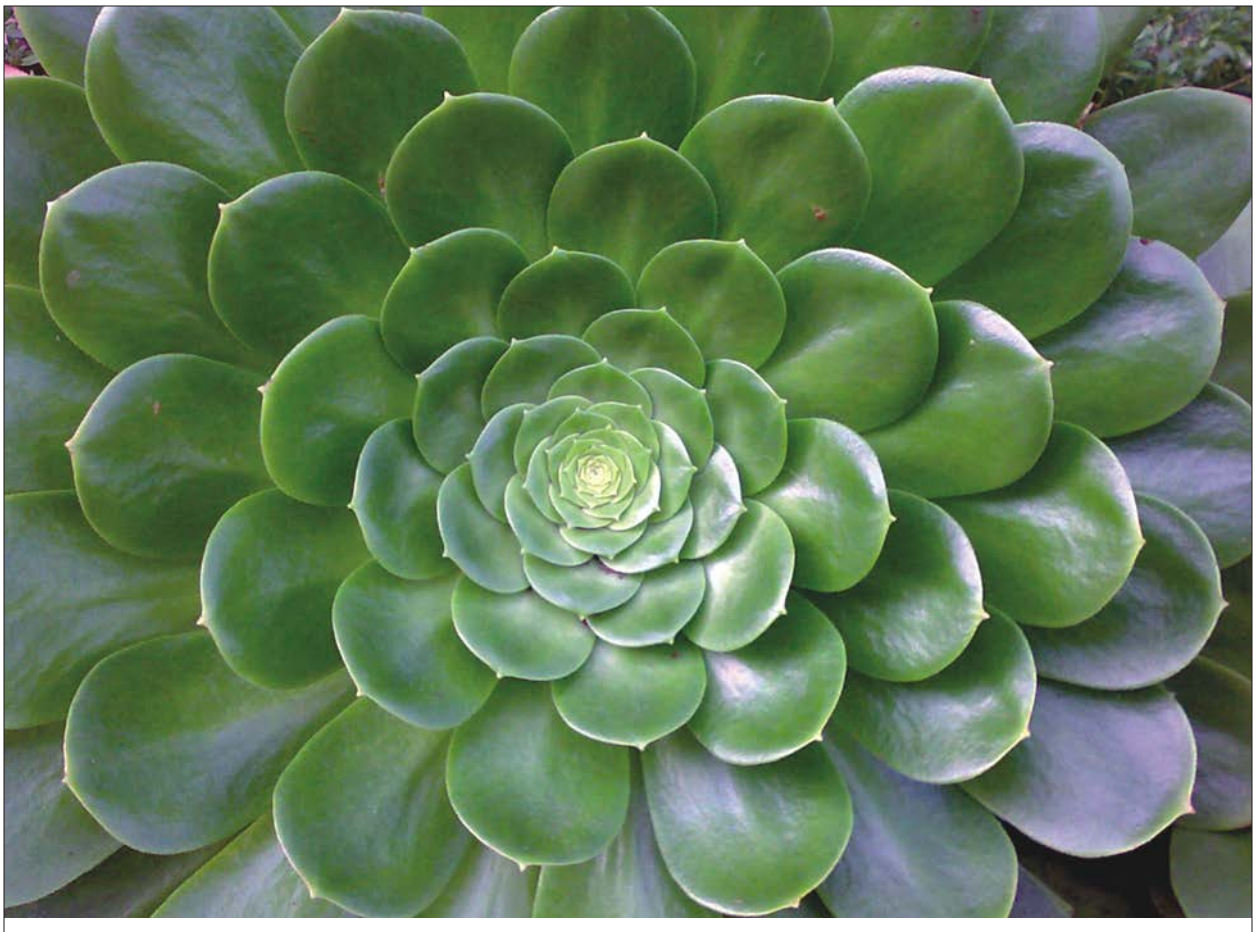

Journal of
Plant Sciences

ISSN 1816-4951

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Journal of Plant Sciences

ISSN 1816-4951
DOI: 10.3923/jps.2016.31.37

Research Article

Callus Induction Studies in *Aquilegia nivalis* Flac Ex Jackson: An Endangered Medicinal Plant of Kashmir Himalaya: A Perspective

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Abstract

Conventional propagation methods take a long time for multiplication because of low rate of growth set or poor germination of the seeds but tissue culture has an important role to play in the manipulation of plants for improved agronomic performance using different concentrations and combinations of plant growth regulator. Murashige and Skoog (MS) media with different combinations and concentrations of growth promoters i.e., auxin (Indole Acetic Acid (IAA), Indole Butyric Acid (IBA)), +synthetic auxin 2, 4-D (2, 4 dichlorophenoxy acetic acid), cytokinin (Benzyl Amino Purine (BAP), +synthetic cytokinin (kinetin (KN) and gibberellin (Gibberalic Acid (GA3). The efficient development of callus type was observed in the MS media supplemented with NAA (2 mg L^{-1}) +kin (2 mg L^{-1}). Three different types of explants viz., leaf (Petiole, middle and tip regions), shoot tips, root and petioles were cultured on MS medium containing 2,4 D, NAA, kinetin and their combinations. Callus was induced from petiole of the leaves of the *Aquilegia nivalis.* Highest and earliest callus formation (29 days) were observed on MS medium containing NAA (1.5 mg L^{-1}) +kinetin (1 mg L^{-1}). Callus proliferation was observed on 2, 4D (2 mg L^{-1}) +kinetin (2 mg L^{-1}) containing medium. Callus induction was not influenced by the season of the explant condition.

Key words: Explants, callus, MS medium, NAA, kinetin, *Aquilegia nivalis*

Received: January 21, 2016 **Accepted:** March 10, 2016 **Published:** April 15, 2016

Citation: Mudasar Ahmad, Zahoor A. Kaloo, B.A. Ganai, Hilal A. Ganaie and Burhan M. Padder, 2016. Callus Induction studies in *Aquilegia nivalis* flac ex Jackson: An endangered medicinal plant of Kashmir Himalaya: A perspective. J. Plant Sci., 11: 31-37.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In view of the tremendously growing world population, increasing anthropogenic activities, rapidly eroding natural ecosystems and the natural habitat for a great number of herbs are dwindling. Many of them are facing extinction. Plant biodiversity is a natural source of products to the medical and food industries. It provides different basic raw materials and contributes to supply new genetic information useful for breeding programs, number of explants for tissue culture and for developing more productive crops and more resistant plants to biological and environmental stresses (Dhar *et al*., 2000; Rao, 2004; Boo *et al*., 2015). To cope up with alarming situation, the recent exciting developments in biotechnology have come as a boon. One of them is the use of plant tissue culture technique (Wani *et al*., 2010; Prakash *et al*., 2014). Most of the plant raised through seeds are highly heterozygous and show great variations in growth, habit and yield and may have to be discarded because of poor quality of products for their commercial release. Likewise, majority of the plants are not amenable to vegetative propagation through cutting and grafting, thus limiting multiplication of desired cultivars. Many plants propagated by vegetative means contain systemic bacteria, fungi and viruses which may affect the quality and appearance of selected items. In recent years, tissue culture has emerged as a promising technique to obtain genetically pure elite populations under *in vitro* conditions rather than have indifferent populations (Reshi *et al*., 2013; Sharma and Vashistha, 2015). Tissue culture has now become a well-established technique for culturing and studying the physiological behavior of isolated plant organs, tissues, cells, protoplasts and even cell organelles under precisely controlled physical and chemical conditions. Most of the medicinal plants either do not produce seeds or seeds are too small and do not germinate in soil. Thus mass multiplication of disease free planting material is a general problem. In this regard, the micro propagation holds significant promise for true to type, rapid and mass multiplication under disease free conditions (Khaliluev *et al*., 2014; Wu *et al*., 2013). Besides, the callus derived plants exhibit huge genetic variation that could be exploited for developing superior clones/varieties particularly in vegetative propagated plant species (Sharma *et al*., 2015).

In vitro callus induction and shoot regeneration has long been employed in plants for both basic biology and applied research including micro-propagation, gene transformation, mutant induction and ploidy manipulation in a number of woody fruit tree such as grape (Mullins and Srinivasan, 1976; Lee and Wetzstein, 1990; Sudarsono and Goldy, 1991), apple (Lane, 1978; Paul *et al*., 1994) and citrus (Gavish *et al*., 1992; Aleza *et al*., 2009; Dutt *et al*., 2010; Kpodar *et al*., 2016).

The Himalaya represent the largest mountain chain in the world, covering about one million sq. km. Himalaya the youngest mountain range of world is famous for its rich plant diversity and varied ecosystem, containing large number of plants. The Trans-Himalayas of Indian cold desert covers under alpine and high alpine zones with peculiar climatic condition featuring and snow covered mountains. The use of plants in curing and healing is as old as mankind (Hedberg, 1987). Plants containing beneficial and medicinal properties have been known and used in some form or other by early people. Biodiversity of Trans-Himalaya is our natural wealth and its conservation is important for economic, ecological, scientific and ethical reasons. Biodiversity provides us with goods and services fundamental to our survival including food, fodder and medicine. The selection of the species used in this study was mainly based on their antibacterial activities. Medicinal plants represent a rich source of antimicrobial and anthelmintic agents (Bhat *et al*., 2012). They are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al*., 1996; Shi *et al*., 2014). Medicinal plants have served through the ages as a constant source of medicaments for the exposure of a variety of diseases (Joshi *et al*., 2013; Jacobo-Herrera *et al*., 2016).

Aquilegia is a genus of perennial herbs, belonging to the Ranunculaceae family, which includes approximately 60-70 species (Sunset Books, 1995). These plants are localized to in the alpine areas of Kashmir Himalaya. *Aquilegia* species are used as the traditional medicine and have a large ethno botanical value e.g., anti-inflammatory, the rhizome of *Aquilegia nivalis* is used against inflammation and wounds, the flowers are used against cough and cold.

MATERIALS AND METHODS

Plant material: The whole plant of *Aquilegia nivalis* flac ex Jackson (Columbine, Zao Neil) were collected from Apharawat area of Gulmarg. The plant was identified by the Plant Taxonomy Division of the Department of Botany, University of Kashmir and submitted the plant specimen with voucher No 1821 Kash Herbarium dated 19-06-2013. Aquilegia is a genus of annual or perennial herbs, belonging to the Ranunculaceae family, which includes approximately 270 species. These plants are localized to Kashmir Himalaya. The *Aquilegia* species occurs as small populations among rocks or in rock crevices on very steep, least stable, moist shady or open slopes at an altitude of 3030-3950 m asl. The present study was conducted during 2015 and is aimed to evaluate callus induction on different hormonal combinations in *Aquilegia nivalis* flac ex Jackson is found in Kashmir (Fig. 1).

Fig. 1: *Aquilegia nivalis* flac ex Jackson: Mother plant

The explants viz., leaf explants (petiole, middle portion and tip), shoot tips and rhizome were sterilized with 2% sodium hypochlorite for 2-6 min and these sterilized explants were cultured on MS medium. The growth regulator combinations were NAA (0.5, 1, 2, 3 and 5 mg L^{-1}), 2, 4, D (0.5, 1, 2, 3 and 4 mg L^{-1}) individually and their combinations with kinetin (1 and 2 mg L^{-1}) and BAP (1 and 2 mg L^{-1}). For seasonal variations, the explants were inoculated every month on MS medium containing NAA (2 mg L^{-1})+kinetin (1 mg L^{-1}) and 2, 4 D (2 mg L^{-1})+kinetin (2 mg L^{-1}) growth regulator combinations.

Sterilization of equipment and glassware's: All processes for *in vitro* culture were execute inside a sterilized horizontal cabinet with HEPA filters. The cabinet surface first sterilized by 70% ethanol using wetted alcohol cotton, then sterilized by an ultraviolet light for at least 15 min prior to use. All tools, glassware and other adnexa were sanitized in autoclave at 121°C with 15 psi for 15 min. Instruments like scalpel, forceps and scissors were sterilized autoclaving and further by dipping in 70% ethanol and flaming prior to use.

Surface sterilization of explants: Young leaves and petioles were placed in a clean beaker and were rinsed under running tap water overnight before the initiation of surface sterilization. The young leaves and petioles were washed with tap water containing 2-3 drops of tween 20 (Qualikems Fine Chem. Pvt. Ltd.) for 10 min. The young leaves and petioles were then rinsed with sterile distilled water several times until all traces of tween 20 were eliminated. The sterilized young leaves were cut into 5×5 mm in size and petioles were cut into 8 cm long pieces transferred to the medium with sterile forceps.

Basal medium: Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) was used as the basal medium. Sucrose at 3% (w/v) was added into the mixture. The pH of the medium was adjusted to 5.7±0.1 with 0.1 M HCl (Sigma) or 0.1 M NaOH (Sigma) followed by addition of 0.8% (w/v) agar. The medium was then autoclaved at 121°C, 15 psi for 15 min. After autoclaving, a total of 15 mL of the sterile medium was poured into culture viols in the laminar flow and was allowed to solidify. The culture viols were then sealed prior to the initiation of treatments.

The callus index was also calculated in *Aquilegia nivalis* by using the equation:

$$\text{Callus index} = \frac{n \times G}{N} \times 100$$

Where:
n = Total number of explants callused
G = Average callus rating on explants
N = Total number of explants cultured

RESULTS

Callus is an unorganized mass of loosely arranged parenchymatous cells. It is the dedifferentiation of a plant cell in to callus. For callus induction, different explants are cultured on nutrient medium supplemented with different concentrations of auxins individually or in combinations with cytokinins. Callus developed from the explants on induction medium is separated and cut into small pieces and transferred to basal medium supplemented with different concentrations of cytokinins individually for shoot initiation. Experiment was conducted to standardize the explant for callusing. Three different types of explants viz., leaf (Petiole, middle portion and tip), shoot tip and rhizome were cultured on MS medium supplemented with growth regulator combinations.

Effect of growth regulators: Highest callus induction percentage (75.67) was recorded on NAA (0.5mg L^{-1})+kinetin (1 mg L^{-1}) followed by NAA (2.3 mg L^{-1})+kinetin (2 mg L^{-1}) treatments (69%) Table 1. The effect of NAA, 2, 4-D and kinetin and their combinations in callus induction revealed that NAA and its combinations with kinetin was more efficient in callus induction. Though callus induction was observed on NAA (0.5 mg L^{-1}) treatments, highest callus induction (75.67%) was recorded on NAA (0.5 mg L^{-1})+kinetin (1 mg L^{-1}). Callus induction was not observed on IAA and its combinations with kinetin.

Table 1: Effect of growth regulators on callus induction (%) and callus response in petiole explant

Concentration of growth regulators (mg L^{-1})			
NAA	Kin	Callus induction (%)	Callus response
1	-	0.00	+
2	-	51.67	+++
3	-	0.00	+
1	1	4.33	++
1	2	5.67	++
2	1	77.67	+++
2	2	69.00	+++
3	1	0.00	+
3	2	0.00	+

+: Low, ++: Moderate, +++: High, NAA: N-acetylasparitic acid and Kin: Kinetin

Table 2: Effect of growth regulators on day taken for callus induction and callus growth

Concentration of growth regulator (mg L^{-1})					
NAA MS Basal	Kin	Days taken for callusing	Relative growth	Callus index	Fresh weight of callus (g)
1.0	-	-	-	-	-
2.0	-	52.50	1.72	88.53	0.74
1.0	1.0	60.00	1.07	4.47	0.57
1.0	2.0	55.75	1.30	7.37	0.64
2.0	1.0	43.25	2.55	200.00	1.85
2.0	2.0	44.50	2.65	181.73	1.94

MS: Murashige and skoog, NAA: N-acetylasparitic and Kin: Kinetin

Table 3: Culture responses in sub culture

		Culture response				
Growth regulator (mg L^{-1})		Sub culturing				
NAA	Kin	BAP	1	2	3	4
MS Basal	-	-	-	-	-	-
2.0	-	-	91.67	90.00	83.33	71.67
2.0	1.0	-	100.00	100.00	100.00	93.33
2.0	2.0	-	100.00	100.00	100.00	93.33
2.0	-	1.0	100.00	100.00	86.67	81.67
2.0	-	2.0	100.00	100.00	100.00	83.33
Mean			98.33	98.00	94.00	84.00

NAA: N-acetylasparitic acid, Kin: Kinetin, BAP: Benzyl amino purine and MS: Murashige and skoog

Table 4: Effect of sub-culturing on callus weight (g)

		Callus weight (g)				
Growth regulator (mg L^{-1})		Sub culturing				
NAA	Kin	BAP	1	2	3	4
MS Basal	-	-	-	-	-	-
2.0	-	-	0.60	0.71	0.82	0.79
2.0	1.0	-	0.93	1.58	2.58	2.37
2.0	2.0	-	1.83	2.88	3.53	2.72
2.0	-	1.0	0.58	0.78	0.81	0.58
2.0	-	2.0	0.68	0.80	0.93	0.67
Mean			0.92	1.35	1.73	1.42

NAA: N-acetylasparitic acid, Kin: Kinetin, BAP: Benzyl amino purine and MS: Murashige and skoog

Callus response: The explants and growth regulator combinations for callusing is scored in Table 1. Profuse callusing was observed on NAA (1.5 mg L^{-1})+kinetin (1 mg mL^{-1}) and NAA (2 mg L^{-1})+kinetin (2 mg L^{-1}) treatments. Earliest response was exhibited on NAA (0.5 mg L^{-1})+kinetin (1 mg L^{-1}) containing medium, where callus was observed within 29 days. This was closely followed by NAA (2 mg L^{-1})+(2 mg L^{-1}) which induced callus in 46.50 days after inoculation. The treatment combinations NAA (1 mg L^{-1})+kinetin (1 mg L^{-1}) took longer time (65.00 days) for callusing. However, highest callus index was observed in (0.5 mg L^{-1})+kinetin (1 mg L^{-1}) containing medium (Table 2).

Sub-culturing: The highest mean culture response 98.33% was found on NAA (2 mg L^{-1})+kinetin (1 mg L^{-1}) (Table 3). The highest culture response was observed in first sub culturing (95.33%) followed by second sub culturing 98.00%. As the number of sub culturing increased, the culture response was found to be decreased. At fourth sub culturing, the culture response was reduced to 84.00. The highest culture response (93.33%) at fourth sub culturing was recorded on NAA (2 mg L^{-1})+kinetin (2 mg L^{-1}) and the lowest response was observed on culture media containing NAA (2 mg L^{-1}). The present study showed that the continuous sub culture resulted in reduction of culture response (Table 3). A gradual decrease in culture response has been observed at each sub culturing at lower concentrations of NAA, kinetin and BAP. However, 100% culture response was maintained up to third subculture on NAA (2 mg L^{-1}) combined with kinetin (1 and 2 mg L^{-1}) and BAP (2 mg L^{-1}) containing medium. Similar results were observed in Callus fresh weight too (Table 4). Highest per cent increase on callus weight were observed in NAA (2 mg L^{-1})+kinetin (2 mg L^{-1}) at third sub culturing and significant reduction was observed in fourth sub culturing.

Influence of season on cultural survival and callus induction: The effect of season was studied by culturing the explant on 2 mg L^{-1}, NAA+1 mg L^{-1} kinetin (K1) and 2 mg L^{-1} NAA+2 mg L^{-1} kinetin (K2) growth regulator media (Table 5 and Fig. 2). The explants were cultured every month and the response were analyzed for the impact of season. The mean culture survival for callus induction was 84.38% in K1 and 83.85% in K2, The callus induction percentage was not influenced by the season in *Aquilegia nivalis* (Fig. 3 and 4).

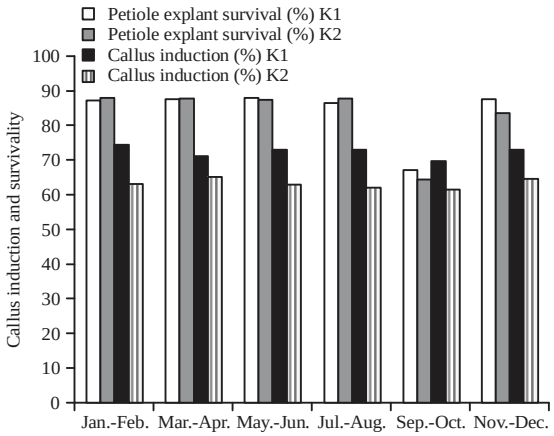

Fig. 2: Effect of month on callus survival and callus induction (%)

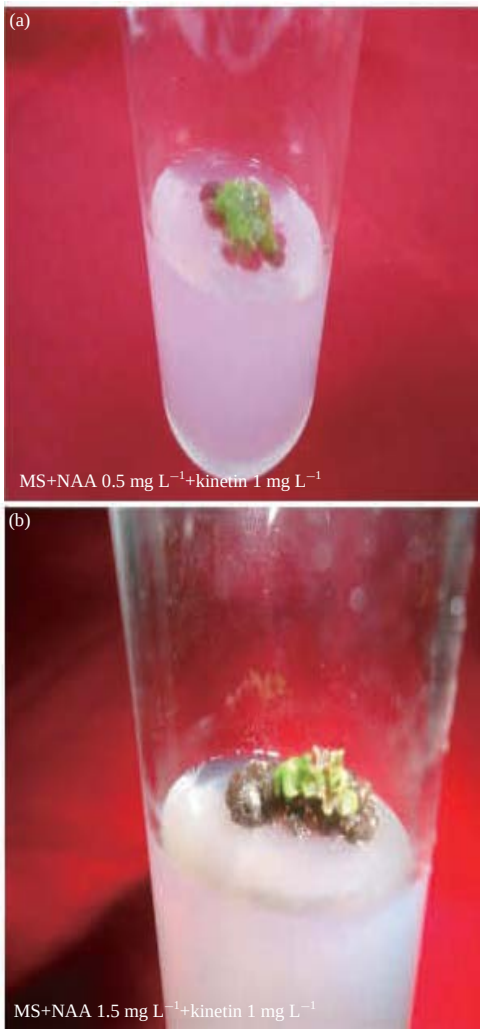

Fig. 3(a-b): Callus induction in *Aquilegia nivalis* on (a-b) MS with NAA (2 mg L^{-1})+kin (2 mg L^{-1}). NAA: N-acetylasparitic acid and MS: Murashige and skoog

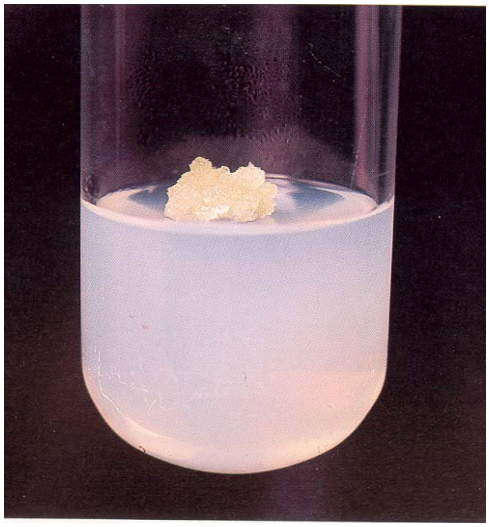

Fig. 4: Callus induction of *Aquilegia nivalis* on different hormonal combinations

Table 5: Effect of season on callus survival and callus induction (%)

	Petiole explant survival (%)		Callus induction (%)	
Seasons	K1	K2	K1	K2
Jan-Feb	87.67	88.33	75.00	63.33
Mar-Apr	88.00	88.33	71.67	65.33
May-Jun	88.33	87.67	73.33	63.33
Jul-Aug	87.00	88.00	73.33	62.33
Sep-Oct	67.33	64.67	70.00	61.67
Nov-Dec	88.00	84.00	73.33	65.00
Mean	84.38	83.85	72.78	63.50
CD	10.21	7.02	NS	NS

NS: Non significant

DISCUSSION

Callus induction could be only obtained from the petiole of leaf explant. The nuclear DNA content of leaf base and tip was lower than the shoot tip and their morphogenetic ability would be less (Sanchez *et al*., 1988; Teshome and Feyissa, 2015). Hence callus formation in this region was difficult. In support of this view reported that no callus was formed in leaf tissue (Lal *et al*., 2002; Sen *et al*., 2014). Callus induction in the petiole portion of leaves might be due to restoration of DNA synthesis favored by hormonal and nutritional conditions available in the callus induction medium (Sanchez *et al*., 1988).

Callus from root tips of *A. saponaria* was also obtained (Yagi *et al*., 1983). In contrast callus was not obtained from the rhizome tips of *Aquilegia nivalis* in the present investigation. The reasons may be due to difference in the genetic constitution of *Aquilegia nivalis,* cultural conditions, nutritional and hormonal status.

Gopi and Vatsala (2006) studied callus and suspension culture from nodal and leaf explants of *Gymnema sylvestre*. Prakash *et al*. (2014) induced callus from nodal and internodal segments of *Crataeva religiosa*. Sharma and Vashistha (2015) developed a protocol for the regeneration of complete plantlets of *Tinospora cordifolia* from the callus induced from leaf explants. Multiple shoot regeneration through a callus phase has been demonstrated in many other woody plants such as *Helicteres isora* (Shriram *et al*., 2008; Upadhyaya *et al*., 2015), *Moringa oleifera* (Kumar *et al*., 2009), *Gmelina arborea* (Kumar *et al*., 2010) and *Morus alba* (Lee *et al*., 2011; Sundram *et al*., 2012).

The results were in confirmation with who reported that among the auxins, NAA was in general more efficient and addition of low concentration of cytokinin will improve the callusing ability of auxin (Bajaj, 2000; Sheeba *et al*., 2013). Higher percentage of callus in leaf explant was also obtained when MS medium supplemented with NAA (0.90 Mm) and kinetin (0.11 Mm) in *Hypercium perforatum* (Bais *et al*., 2002). The loss of morphogenetic potential in continuous sub cultures might be due to low levels of endogenous hormones (Wochok and Wetherell, 1972). The above observation is in confirmation with the findings of in *Gloriosa superba* and also in *Phyllanthus amarus* (Chitra, 2001).

Lowest survival percentage of 67.33 and 64.67, respectively in K1 and K2 was obtained in September-October such effects in explant was observed was observed in *Gloriosa superba* and in *Phyllanthus amarus* (Chitra, 2001).

CONCLUSION

An efficient protocol was developed for successful callus induction in *Aquilegia nivalis* from three different explants viz., leaf, petiole and intermodal cuttings. The MS medium supplemented with different plant growth regulators were used. Among all the explants, petiole explants proved to be more responsive as they produced the maximum amount of callus in less number of days when inoculated on MS medium containing NAA and kinetin (KN).

ACKNOWLEDGMENTS

Authors are highly thankful to the Head, Department of Botany, University of Kashmir, Srinagar, for providing necessary facilities and also to Curator, Centre of Plant Taxonomy, Department of Botany, University of Kashmir for identifying the plant.

REFERENCES

Aleza, P., J. Juarez, P. Ollitrault and L. Navarro, 2009. Production of tetraploid plants of non apomictic citrus genotypes. Plant Cell Rep., 28: 1837-1846.

Bais, H.P., T.S. Walker, J.J. McGrew and J.M. Vivanco, 2002. Factors affecting growth of cell suspension cultures of *Hypericum perforatum* L. (St. John's wort) and production of hypericin. *In vitro* Cell. Dev. Biol.-Plant, 38: 58-65.

Bajaj, Y.P.S., 2000. Micro Propagation of *Aegle marmelos*. In: High-Tech and Micropropagation, Bajaj, Y.P.S. (Ed.). Springer, New York, USA., pp: 281.

Bhat, P., G. Hegde and G.R. Hegde, 2012. Ethnomedicinal practices in different communities of Uttara Kannada district of Karnataka for treatment of wounds. J. Ethnopharmacol., 143: 501-514.

Boo, K.H., D.V. Cao, R.S. Pamplona, D. Lee, K.Z. Riu and D.S. Lee, 2015. *In vitro* plant regeneration of *Aster scaber* via somatic embryogenesis. Biosci. Biotechnol. Biochem., 79: 725-731.

Chitra, R., 2001. *In vitro* studies in *Phyllanthus amarus*. M.Sc. Thesis, Faculty of Horticultural Science and Institutes of Tamil, Nadu Agricultural University.

Dhar, U., R.S. Rawal and J. Upreti, 2000. Setting priorities for conservation of medicinal plants-a case study in the Indian Himalaya. Biol. Conserv., 95: 57-65.

Dutt, M., M. Vasconcellos, K.J. Song, F.G. Gmitter Jr. and J.W. Grosser, 2010. *In vitro* production of autotetraploid Ponkan mandarin (*Citrus reticulata* Blanco) using cell suspension cultures. Euphytica, 173: 235-242.

Gavish, H., A. Vardi and R. Fluhr, 1992. Suppression of somatic embryogenesis in Citrus cell cultures by extracellular proteins. Planta, 186: 511-517.

Gopi, C. and T.M. Vatsala, 2006. *In vitro* studies on effects of plant growth regulators on callus and suspension culture biomass yield from *Gymnema sylvestre* R. Br. Afr. J. Biotechnol., 5: 1215-1219.

Hedberg, I., 1987. Research on Medicinal and Poisonous Plants of Tropics: Past, Present and Future. In: Medicinal and Poisonous Plants of the Tropics, Leeuwenberg, A.J.M. (Ed.). Pudoc, Dehra Dun, India, ISBN-13: 9789022009215, pp: 9-15.

Jacobo-Herrera, N.J., F.E. Jacobo-Herrera, A. Zentella-Dehesa, A. Andrade-Cetto, M. Heinrich and C. Perez-Plasencia, 2016. Medicinal plants used in Mexican traditional medicine for the treatment of colorectal cancer. J. Ethnopharmacol., 179: 391-402.

Joshi, A., N. Sengar, S.K. Prasad, R.K. Goel, A. Singh and S. Hemalatha, 2013. Wound-healing potential of the root extract of *Albizzia lebbeck*. Planta Medica, 79: 737-743.

Khaliluev, M.R., L.R. Bogoutdinova, G.B. Baranova, E.N. Baranova, P.N. Kharchenko and S.V. Dolgov, 2014. Influence of genotype, explant type and component of culture medium on *in vitro* callus induction and shoot organogenesis of tomato (*Solanum lycopersicum* L.). Biol. Bull., 41: 512-521.

Kpodar, M.S., S.D. Karou, G. Katawa, K. Anani and H.E. Gbekley *et al*., 2016. An ethnobotanical study of plants used to treat liver diseases in the Maritime region of Togo. J. Ethnopharmacol., 181: 263-273.

Kumar, A., M.D.S. Ahmad and M.D. Naseem, 2010. *In vitro* plant regeneration from organ cultures of *Gmelina arborea* Roxb. J. Indian Bot. Soc., 8: 197-203.

Kumar, U., I. Singh and Y. Vimala, 2009. *In vitro* regeneration of *Moringa oleifera*. J. Indian Bot. Soc., 88: 120-123.

Lal, R.K., J.R. Sharma, D.D. Patara, A.H.A. Farooqui and A. Kalra *et al*., 2002. *Aloe vera* and its cultivation in India. Farm Bulletin No. 1, Central Institute of Medicinal and Aromatic Plants, Lucknow, India, pp: 1-15.

Lane, W.D., 1978. Regeneration of apple plants from shoot meristem-tips. Plant Sci. Lett., 13: 281-285.

Lee, N. and H.Y. Wetzstein, 1990. *In vitro* propagation of muscadine grape by axillary shoot proliferation. J. Am. Soc. Horticult. Sci., 115: 324-329.

Lee, Y., D.E. Lee, H.S. Lee, S.K. Kim, W.S. Lee, S.H. Kim and M.W. Kim, 2011. Influence of auxins, cytokinins and nitrogen on production of rutin from callus and adventitious roots of the white mulberry tree (*Morus alba* L.). J. Plant Cell Tissue Org. Cult., 105: 9-19.

Mullins, M.G. and C. Srinivasan, 1976. Somatic embryos and plantlets from an ancient clone of the grapevine (cv. Cabernet-Sauvignon) by Apomixis *in vitro*. J. Exp. Bot., 27: 1022-1030.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum, 15: 473-497.

Paul, H., M. Belaizi and B.S. Sangwan-Norreel, 1994. Somatic embryogenesis in apple. J. Plant Physiol., 143: 78-86.

Prakash, A., S. Kumari, Utkarshini, K. Sinha and S. Kumar, 2014. Direct and callus mediated regeneration from nodal and internodal segments of *Crataeva religiosa* G. Forst. var. nurvala (Buch.-Ham.) Hook. f. and Thomson. Indian J. Biotechnol., 13: 263-267.

Rao, N.K., 2004. Plant genetic resources: Advancing conservation and use through biotechnology. Afr. J. Biotechnol., 3: 136-145.

Reshi, N.A., M.S. Sudarshana and N. Rajashekar, 2013. Callus induction and plantlet regeneration in *Orthosiphon aristatus* (blume) Miq.-a potent medicinal herb. IOSR J. Pharm. Biol. Sci., 6: 52-55.

Sanchez, I.C., L. Natali and A. Cavallin, 1988. *In vitro* culture of *Aloe barbadensis* Mill.: Morphogenetic ability and nuclear DNA content. Plant Sci., 55: 53-59.

Sen, M.K., S. Nasrin, S. Rahman and A.H.M. Jamal, 2014. *In vitro* callus induction and plantlet regeneration of *Achyranthes aspera* L., a high value medicinal plant. Asian Pac. J. Trop. Biomed., 4: 40-46.

Sharma, H. and B.D. Vashistha, 2015. Plant tissue culture: A biological tool for solving the problem of propagation of medicinally important woody plants-A review. Int. J. Adv. Res., 3: 402-411.

Sharma, S., B. Kamal, N. Rathi, S. Chauhan and V. Jadon *et al*., 2015. *In vitro* rapid and mass multiplication of highly valuable medicinal plant *Bacopa monnieri* (L.) Wettst. Afr. J. Biotechnol., 9: 8318-8322.

Sheeba, E., S. Palanivel and S. Parvathi, 2013. Effect of plant growth regulators on callus induction in *Physalis minima* Linn. Int. J. Innov. Res. Sci., 2: 4847-4851.

Shi, H., Y. Zhu, B. Wang, J. Sun and S. Huang, 2014. [Induction and *in vitro* culture of hairy roots of *Dianthus caryophyllus* and its plant regeneration]. Sheng Wu Gong Cheng Xue Bao, 30: 1742-1750, (In Chinese).

Shriram, V., V. Kumar and M.G. Shitole, 2008. Indirect organogenesis and plant regeneration in *Helicteres isora* L., an important medicinal plant. *In vitro* Cell. Dev. Biol.-Plant, 44: 186-193.

Srivastava, J., J. Lambert and N. Vietmeyer, 1996. Medicinal Plants: An Expanding Role in Development. World Bank, Washington DC, USA., pp: 320.

Sudarsono and R.G. Goldy, 1991. Growth regulator and axillary bud position effects on *in vitro* establishment of *Vitis rotundifolia*. HortScience, 26: 304-307.

Sundram, T.C.M., M. Suffian, M. Annuar and N. Khalid, 2012. Optimization of culture condition for callus induction from shoot buds for establishment of rapid growing cell suspension cultures of Mango ginger (*Curcuma mangga*). Aust. J. Crop Sci., 6: 1139-1146.

Sunset Books, 1995. Sunset Western Garden Book. 6th Edn., Sunset Publishing Corporation, Oakland, CA., USA., ISBN: 9780376038517, pp: 606-607.

Teshome, S. and T. Feyissa, 2015. *In vitro* callus induction and shoot regeneration from leaf explants of *Glinus lotoides* (L.)-an important medicinal plant. Am. J. Plant Sci., 6: 1329-1340.

Upadhyaya, G., M. Sen and A. Roy, 2015. *In vitro* callus induction and plant regeneration of rice (*Oryza sativa* L.) var. 'Sita', 'Rupali' and 'Swarna Masuri'. Asian J. Plant Sci. Res., 5: 24-27.

Wani, M., S. Pande and N. More, 2010. Callus induction studies in *Tridax procumbens*, L. Int. J. Biotechnol. Appl., 2: 11-14.

Wochok, Z.S. and D.F. Wetherell, 1972. Restoration of declining morphogenetic capacity in long term tissue cultures of *Daucus carota* by kinetin. Experientia, 28: 104-105.

Wu, Q.G., L.J. Zou and M.H. Luo, 2013. [Callus induction and plant regeneration of *Sambucus chinensis*]. Zhong Yao Cai, 36: 1899-1903, (In Chinese).

Yagi, A., Y. Shoyama and J. Nishioka, 1983. Formation of tetrahydroanthracene glucosides by callus tissue of *Aloe saponaria*. Phytochemistry, 22: 1483-1484.